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SOME PROPERTIES OF THE TRICHLORACETIC ACID EXTRACT OF *BR. ABORTUS*, WITH SPECIAL REFERENCE TO IMMUNISATION

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The chemical fractionation of Brucella organisms has been studied by a number of workers, notably Huddleson and his colleagues (Huston, Huddleson and Hershey, 1934; Hershey, Huddleson and Pennell, 1935; Hershey and Huddleson, 1936; Pennell and Huddleson, 1937; Stahl, Pennell and Huddleson, 1939) and more recently Miles and Pirie (1939a, b). It is no part of this work to make a critical analysis of these studies; they are mentioned only to indicate certain work that has been done on the preparation of antigenic extracts of Brucella organisms. At the same time, it may be noted that, as yet, relatively little attention has been paid to the possibility of immunisation with such fractions. In this connection, Roman (1938) has claimed that guinea-pigs can be given a high degree of protection against *Br. melitensis* infection by the simultaneous inoculation of an extract of *Br. melitensis* and a suspension of living, non-virulent, smooth *Br. abortus* organisms; later (Lisbonne, Roman and Renoux, 1939) this work was extended to sheep with a comparable result. The purpose of the work reported in this paper was to see if a similar immunity could be built up in guinea-pigs to *Br. abortus* infection.

It is necessary here to refer briefly to the work of Boivin and Mesrobeanu (1935, 1937, 1938) since Roman adopted their technique for the preparation of his extracts of *Br. melitensis*. These workers found that bacterial somatic antigens are contained in a trichloracetic acid extract of the organisms. The antigen is reputed to be non-protein in character and to consist of a polysaccharide attached to a radical composed of fatty acids. The polysaccharide, which can be split off by mild hydrolysis, is a hapten and non-toxic. Boivin and Mesrobeanu give details of methods whereby a number of impurities can be removed; these methods consist mainly of dialysis and precipitation with acetone or alcohol.

This work has been confined to three strains of *Br. abortus, viz.* strains 45, 45 (20) and 161. Details of the virulence and toxicity of these strains have been given by McEwen (1940, see also McEwen and Roberts, 1936; McEwen and Priestley, 1938; Priestley and McEwen, 1938; Priestley, 1939); here it is sufficient to mention that strain 45 is completely non-virulent and smooth; strain 45 (20) is a variant of 45 and its virulence has been raised by passage through a series of 20 guinea-pigs, becoming rough, as judged by the tests of Pandit and Wilson (1932) during this passage; and strain 161 is fully virulent and smooth. All these strains are aerobic.

Since the method adopted for the preparation of the extract was essentially similar to that of Boivin and Mesrobeanu no details need be given here.

GENERAL PROPERTIES OF THE NEUTRALISED EXTRACT

REACTION WITH ANTISERUM.

Extracts from smooth strains precipitate specifically with homologous antiserum and titration by this means appears to be the most satisfactory method for quantitative estimations.

It is essential to use a highly potent serum. Such sera may be prepared in rabbits by a series of intravenous inoculations of relatively thin suspensions of dead organisms, followed by one massive dose. Sera prepared in this way will react with antigen when diluted as much as 1 in 5.

The titre of the antigen varies somewhat from batch to batch. In general, extract from strain 161 reacts at a dilution of 1 in 200, and extract from strain 45 at a dilution of about 1 in 10. These dilutions, as will be seen later, correspond to a final dilution of acetone precipitable substance of about 1 in 160,000. If this antigen is chemically anything like that isolated by Miles and Pirie from *Br. melitensis*, the extract must contain a large amount of impurity since these workers obtained end-titres as high as 1 in $2{\cdot}5 \times 10^6$.

The material reacting with serum is heat-stable, resisting 100° C. for at least 30 mins., *i.e.*, extracts heated in neutral solution in this manner show no diminution of end-titre.

Extract from strain 45 (20) fails to react with "smooth" sera. Whether this is due to absence of antigen or to the presence of a serologically distinct antigen, has not been determined. All attempts to obtain a "rough" precipitating serum have failed; it would seem, therefore, that R strains have little or no extractable antigen.

ANTIGENICITY.

That the extract of S strains is antigenic has been shown several times. The following experiment was a typical one. An extract of strain 161 was prepared in the usual way; some of the extract was

filtered through a Seitz filter and some of the filtrate was heated in a boiling water bath for 30 minutes. 0·5 c.c. amounts of these three solutions were inoculated intravenously into separate rabbits; a second inoculation of 5·0 c.c. was given five days later and the rabbits were bled and tested seven days after this second inoculation. Sera from all rabbits before inoculation contained no agglutinins. After inoculation, the serum from the rabbit receiving unfiltered, unheated extract had an agglutinin titre greater than 1 in 1,024; serum from the rabbit receiving filtered, unheated extract had a titre of 1 in 512; serum from the rabbit receiving filtered and heated extract had a titre of 1 in 1,024; and all sera precipitated with extract.

Thus this extract is antigenic and heat-stable.

Toxicity

The neutralised extract does not appear to be so markedly toxic for mice as are similar extracts from other bacteria.

In one experiment, an extract was prepared in the usual way and filtered. Various dilutions in saline were prepared and 1·0 c.c. amounts were injected intraperitoneally into batches of six mice. The first dilution used had an end-titre with serum of 1 in 50; it was calculated that in each c.c. there was present 0·3125 mgm. acetone-precipitable material. This dilution killed five out of six mice within 24 hours; the sixth mouse and all those inoculated with lesser amounts of extract survived for seven days without showing any signs of illness.

In another experiment of this kind, in which the results were similar, there were 12 survivors. These mice were tested for immunity to the toxic action of the whole bacteria. Nine days after the primary inoculation, the 12 surviving animals, together with five normal mice were inoculated intraperitoneally with 1·0 c.c. of a living suspension of a fully virulent and toxic strain (544) standardised to tube 7 on the Brown Scale. Further groups of normal mice were inoculated with suspension equivalent in density to ½, ¼ and ⅛ the original. The results after four days were as follows:—

Mice Receiving Suspension.	Equivalent to Tube.	Died.	Per cent.
Of 12 immunised	7	4	33·3
„ 5 normal	7	5	100
„ 5 „	3·5	4	80
„ 5 „	1·75	3	60
„ 5 „	0·875	0	0

Thus, immunisation with the extract protected a proportion of mice against about four lethal doses.

Allergy.

Neutralised extract, at least in its original unconcentrated state, fails to elicit any skin reaction in infected guinea-pigs. 0·1 c.c. inoculated intradermally causes a very slight reddening for a day or two. The same reaction occurs in normal guinea-pigs and necrosis or marked inflammation is not observed.

Precipitation with Acetone.

If to a measured volume of neutralised extract are added about three volumes of acetone, a flocculent precipitate forms which can be collected by centrifuging. This precipitate contains the antigen. If it is washed once or twice in acetone and then in ether, it can be dried and weighed.

The yield of acetone-precipitable substance varies very markedly from one batch of suspension to another, depending to a large extent on how thoroughly the bacteria were washed prior to treatment. There is no doubt that this variation is due to impurities derived from the medium, since a precipitate of the same sort can readily be obtained if unsown agar is washed down with distilled water or saline. It follows, therefore, that the weight of precipitate obtained is no measure of the antigen present.

To give some idea of the yield from well-washed bacteria, the following experiment is quoted. Suspensions of strains 161 and 45 were centrifuged and washed in three changes of distilled water and were ultimately suspended in distilled water so that each c.c. contained 50 mgm. dried bacteria. These suspensions were then treated with trichloracetic acid, centrifuged and neutralised in the usual way. 80 c.c. of the neutralised extract of strain 161 gave 100 mgm. of acetone precipitable material, a yield of 5 per cent. from the dried bacteria. The end-titre of this extract with serum was 1 in 200. The primary dilution of acetone precipitable material was 1 in 800 (1·25 mgm. per c.c.); thus the end-titre of this material was 1 in 160,000. Similarly, 80 c.c. of neutralised extract of strain 45 gave approximately 50 mgm., a yield of 2·5 per cent. The end-titre of the extract lay between 1 in 10 and 1 in 100; thus the end-titre of this acetone precipitable material approximated to that of strain 161.

A smaller amount of precipitate may be obtained from the rough strain 45 (20); as remarked before, whether this represents rough antigen or not remains for the moment unknown.

IMMUNISATION EXPERIMENTS

Experiment 1.

The object of this experiment was to compare the immunising abilities of (*a*) a suspension of a non-virulent strain of *Br. abortus*, (*b*) neutralised trichloracetic acid extract from a virulent strain of *Br. abortus* and (*c*) a mixture of (*a*) and (*b*).

Accordingly, four groups of guinea-pigs were formed. Each guinea-pig in Group 1 received intramuscularly 1·0 c.c. of a mixture of equal parts of saline and of a saline suspension of a two-day growth of the non-virulent strain 45 standardised by opacity to tube 6 of the Brown Scale. Group 2 similarly received a mixture of equal parts of saline and a saline solution containing 1·0 mgm. per c.c. of the acetone precipitable material from extract of the virulent strain 161. Group 3 received 1·0 c.c. of a mixture of equal parts of a suspension of strain 45 and of the solution of acetone precipitable material. Group 4 was uninoculated.

Seven days later the animals received a second inoculation similar to the first except that the solution of acetone precipitable material now contained 2 mgm. per c.c.

Seven days later again, the animals were given a final inoculation similar to the preceding except that the solution of the acetone precipitable material now contained 5 mgm. per c.c.

Fourteen days after the last immunising dose had been given, each group of animals was split up into three sub-divisions and infected by placing on the

surface of the eye one drop of a suspension of the fully virulent CO^2-sensitive strain 544. Three infecting doses were used as indicated in Table I.

Thirty-two days after infection, all the animals were killed and their spleens cultured on to liver agar. At the same time, note was taken of any gross abnormalities and serum was tested for agglutinin content. In nearly all cases the spleen was slightly enlarged and in nearly all those animals which were positive to culture, the serum had an agglutinin titre of 1 in 160 or over. The results of spleen culture are shown in Table I.

It will be noted that with the two highest infecting doses, there was 100 per cent. infection in all groups. With the smallest dose, the degree of infection was rather less. It is clear that none of the immunising agents has given any degree of protection.

Table II

The Cutaneous Titration of Strain 544

Infecting Dose 0·1 c.c.

Dilution of Suspension.	No. of Organisms Inoculated.	No. of Animals Exposed.	No. of Animals Infected.
Undiluted ...	500×10^6	3	3
Diluted—			
1 in 10 ...	50×10^6	1	1
1 in 10^2 ...	5×10^6	3	3
1 in 10^3 ...	5×10^5	3	2
1 in 10^4 ...	5×10^4	3	2
1 in 10^5—1 in 10^7	5×10^3—50	3	0

Table I

Showing the Immunising Effect of Non-virulent Br. abortus *Organisms and Precipitate from Extract of a Virulent Strain.*

Immunising Agent.	Infecting Dose: Strain 544 Standardised to Tube 3 Brown Scale.					
	Undiluted.		Diluted 1 in 10.		Diluted 1 in 100.	
	No. Exposed.	No. Infected.	No. Exposed.	No. Infected.	No. Exposed.	No. Infected.
Strain 45	12	12	12	12	12	8
Extract ppt.	12	12	11	11	12	11
Strain 45 + Extract ppt. ...	12	12	12	12	12	10
	12	12	12	12	12	8

Experiment 2 (Titration of Infective Dose).

Since the first immunisation experiment was so unsuccessful, it was decided to repeat the work following the technique of Roman in principle as exactly as possible.

To do this, it was first of all necessary to titrate an infective dose by the cutaneous route. Accordingly the hair was clipped off the backs of a number of guinea-pigs and an area shaved until light scarification was produced. 0·1 c.c. of various dilutions of the infecting organisms were then placed on these areas. The virulent strain, 544, was the one chosen for titration. A two-day growth on liver agar was washed down with sterile saline and the suspension standardised to tube 3 of the Brown Scale; consecutive 1 in 10 dilutions were made up to 1 in 10^7. The undiluted suspension contained approximately $5{,}000 \times 10^6$ viable organisms per c.c. 0·1 c.c. of this suspension therefore contained about 500×10^6 organisms.

Eight weeks later the animals were killed and examined in the usual way. The results of spleen culture are shown in Table II. It may be noted that, with 5×10^6 organisms or more, every animal exposed was infected and that with 5,000 organisms or less every animal exposed escaped infection.

For the infection of immunised animals, it was decided to employ a dose of 5×10^6 organisms, *i.e.*, 0·1 c.c. of a 1 in 100 dilution of a suspension standardised by opacity to tube 3 of the Brown Scale. This would appear to be an infective dose of the same order as that used by Roman; his dose was actually stated to be 2×10^6 organisms and 200 times the minimal infecting dose.

Experiment 2 (Immunity Test).

The extract for this experiment was prepared from fresh, moist and thoroughly washed organisms, the final neutralised product being filtered through a Seitz filter and stored in the ice-chest. It was a potent extract reacting specifically with a positive serum at a dilution of 1 in 100 and giving no reaction with a negative serum.

Six groups of animals were formed as shown in Table III. Each immunised animal received a total inoculum of 1·5 c.c., 0·5 c.c. being given on three occasions with intervals of seven days. All inoculations were done intradermally on the back.

The animals in Group 1 received, on each occasion, 0·5 c.c. of a mixture of two parts saline and one part of a 1 in 10 dilution of a suspension of the non-virulent strain 45, standardised to tube 3 of the Brown Scale; this inoculum contained approximately 50×10^6 viable organisms.

The animals in Group 2 similarly received 0·5 c.c. of a mixture of two parts neutralised extract and one part saline.

Group 3 animals received 0·5 c.c. of a mixture of two parts neutralised extract and one part suspension of the non-virulent strain 45.

Group 4 animals received 0·5 c.c. of a mixture of two parts neutralised N/4 trichloracetic acid and one part saline.

Group 5 animals received 0·5 c.c. of a mixture of two parts neutralised acid and one part suspension of strain 45. Groups 4 and 5 were included to check the possibility that any resulting immunity was due to non-specific effects.

Group 6 animals received no primary inoculation and acted as controls of the infection.

Unfortunately, during the course of this experiment, some intercurrent infection caused the death of a number of animals, very considerably reducing the numbers available in some groups. However, four weeks after the last dose of vaccine, all surviving animals were infected on the scarified skin of the back in the manner indicated above, with approximately 5×10^6 viable organisms of the virulent strain 544.

Eight weeks after infection, all animals were killed and examined. At *post-mortem* examination, it was noted that, in nearly every case, the spleen was definitely enlarged, and the sera of all animals were strongly positive to the agglutination test. Spleen cultures gave growth of *Br. abortus* in every case. In other words, since the vaccine strain is most unlikely to have survived in the tissues for 12 weeks (see McEwen, 1940) every animal was infected and no immunity was demonstrated.

Table III

Showing the Immunising Effect of Extract of a Virulent Strain and Suspension of a Non-virulent Strain of Br. abortus

Group.	Immunising Agent.	Nos. Available at Conclusion of Experiment.	Nos. Infected.
1	Strain 45	1	1
2	161 extract	5	5
3	Strain 45 and 161 extract	8	8
4	Neutralised trichloracetic acid	5	5
5	Neutralised acid and strain 45	2	2
6	—	6	6

Discussion and Summary

Following the technique of Boivin and Mesrobeanu, trichloracetic acid extracts of *Br. abortus* have been prepared and examined. The active principle of such extracts reacts specifically with serum, is antigenic, heat-stable, precipitable with acetone, non-dialysable through cellophane membranes and slightly toxic. It fails to elicit any skin reaction in infected guinea-pigs. Such a substance is extractable from " smooth " types of *Br. abortus* whether these be virulent or non-virulent. All attempts to extract a similar substance from a rough strain have failed completely. For these and other reasons, it is assumed, therefore, that the active principle contains the smooth somatic antigen.

Unsuccessful attempts were made to protect guinea-pigs against *Br. abortus* infection, using a mixed vaccine consisting of a suspension of viable non-virulent *Br. abortus* organisms and a trichloracetic acid extract of virulent organisms.

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ANIMALS' REACTIONS TO WAR

A Special Correspondent of *The Times* writes as follows in a recent issue of that paper: " Some time ago your correspondent recorded the escapades of Billy, a tame European badger, who escaped from his cage, and, though no air raid warning had been given, made a dive straight for one of the tunnels that have been converted into an air raid shelter in Regent's Park; he was found hiding there after a long search.

" Billy's liking for air raid shelters appears, however, to have been eclipsed by a great-crested grebe which was found in a public shelter at Euston during a raid, and was brought to the Zoo by the police. It appears to be suffering from its experience, but the staff of the Bird House is striving to nurse it back to health. No possible stretch of ornithological imagination could have suggested air raid shelters as a habitat for this bird of lakes and still waters.

" Much comment has appeared in the newspapers about the reactions of animals, both inside and outside the Zoo, to air raids, bombs, and sirens. Tales of mass migrations of small birds from the 'hot' areas of the South-East Coast should be examined very carefully before any of them can be accepted; Mr. H. S. Gladstone's book about birds in the last Great War shows that on the whole they take very little notice of bombardment; certainly there is no evidence that wild or captive creatures in London have noticed the *Blitzkrieg* to any more significant extent than its human inhabitants have. There is certainly no evidence that any of the animals in the Zoo can (*a*) anticipate the arrival of aircraft, (*b*) distinguish between ours and theirs, (*c*) supplement, or react in any important way to the sirens, or (*d*) foresee the impact of bombs.

" The animals are, however, not entirely neutral to disturbances. After a few minutes of anti-aircraft fire the other night the cranes got rather excited and began their rattling cries; but they soon settled down. The gibbons have a cry that resembles the siren in some ways (though nobody who lives near the Zoo fails to distinguish them); they are so accustomed to striking up their chorus at odd moments of the day or night that it would be unfair to attribute any significance to the coincidence of their song with any of the alarms or excursions of war.

" Your correspondent would welcome any concise first-hand accounts of the reactions of any creatures, wild or captive, to the affairs and noises of war. These can be addressed to the Zoological Society, N.W.8, and will be acknowledged as soon as is humanly possible."

* * * * *

The Rockefeller Foundation has donated £1,000 to the Royal Society of Medicine for the purpose of paying the expenses of moving a large number of valuable books from the London headquarters to a suitable place in the country.

AN AUTOMATIC SWITCH FOR ELECTRIC CENTRIFUGES

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The switch described in this note replaces that operated by a clock which I described in the *Journal of Comparative Pathology and Therapeutics* in 1937. That mechanism worked satisfactorily for about four years, but a tendency developed for the trigger to stop the minute hand of the clock, instead of the hand operating the trigger. This was due to weakening of the spring on the spindle of the minute hand which couples that spindle to the clock mechanism.

To overcome this defect it was necessary to replace this spring-controlled pressure by a positive pressure, and the idea presented itself that the revolution of the centrifuge might be utilised for the purpose. As the machine to which this mechanism has been fixed is used for the sedimentation of milk samples in connection with the detection of tubercle bacilli, only one standard period of running had to be arranged for.

The problem was to devise some means whereby the spindle of the centrifuge could be made to actuate a revolving arm so that it would make one revolution only during the period of running of the machine. This was achieved by using a series of spindles carrying worm and gear wheels. The first worm wheel is fixed to the top of the centrifuge spindle and the remainder of the wheels on the under or upper side of the lid of the machine, the position being determined by the amount of space available under the lid. The lid must be hinged to ensure correct location.

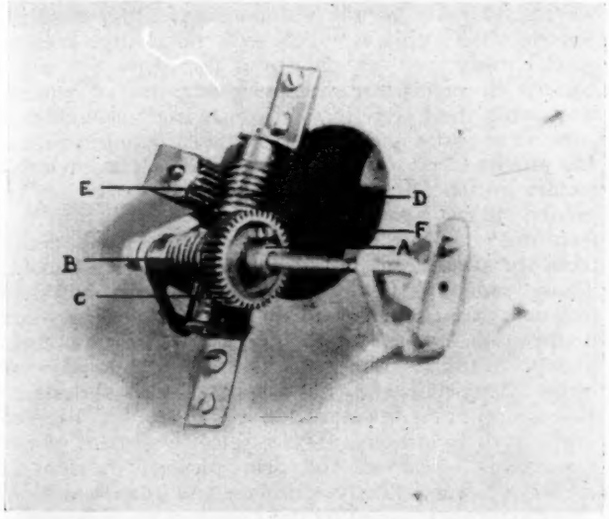


FIG. I.

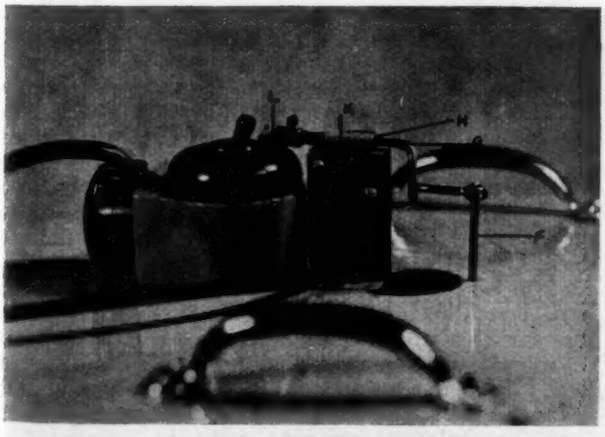


FIG. II.

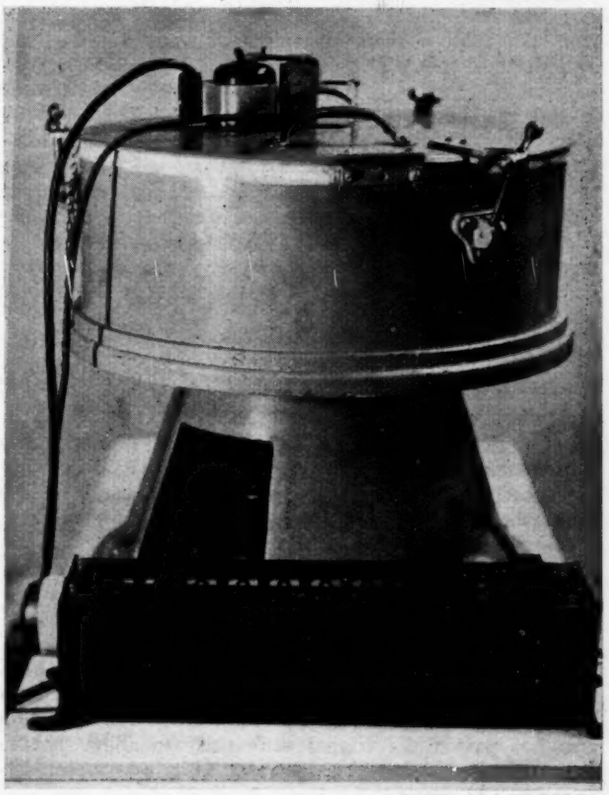


FIG. III.

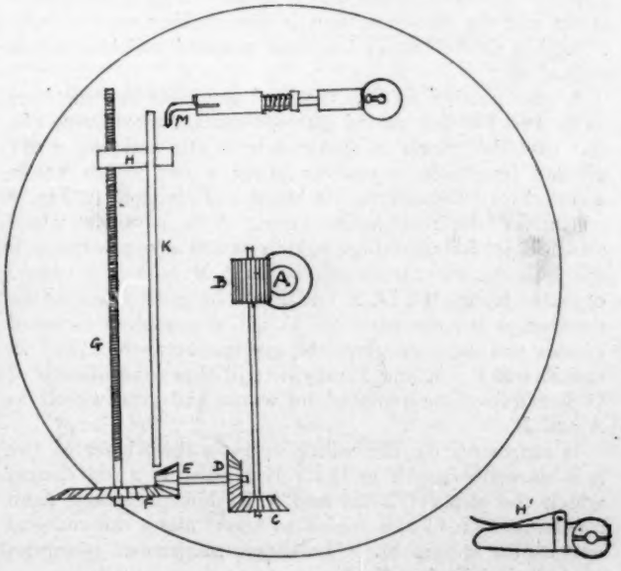


FIG. IV.

The worm wheel on the centrifuge spindle engages a gear wheel on the spindle of which is a second worm wheel. These are shown at A and B in Fig. 1. The worm wheel B engages another gear wheel C which has on its spindle a third worm wheel D. This, in turn, engages a gear wheel E, the spindle of which passes through the lid of the machine and carries on its upper end an arm for operating the trigger switch. (Fig. 2.F.)

The trigger (Fig. 2.G) is a rod bent at right angles running through a stout-walled tube which is slotted at one end (Fig. 2.K). About three-quarters of an inch from the angle a short crosspiece of stout wire is fixed into the rod (H). The other end of the rod carries an adjustable plunger (L) with a compression spring between it and the tube. When the spring is compressed the wire crosspiece passes out of the slot and by slight rotation of the trigger is brought against the end of the tube, thus keeping the spring compressed. As the operating arm (F) travels round, it comes into contact with the trigger and causes it to rotate in the tube. When the crosspiece reaches the slot, the spring expands suddenly and causes the plunger to strike the knob of the switch and turn it off.

It is a simple matter to determine what gear wheels will be required for any particular period of running if the speed of the machine fully loaded is known. As one revolution of a worm wheel moves a gear wheel in engagement with it only one tooth, the speed of the spindle carrying the gear wheel is obtained by dividing the speed of the machine by the number of teeth on the gear wheel. The reduction given by the second and third worm wheels is ascertained in the same way. Combinations of gears to give any required time of running for a given speed of the centrifuge can be worked out.

In calculating what gears are required, some allowance must be made for the machine to work up to full speed, and also for running down after the switch has been turned off. It is not necessary to leave the lid closed until the machine is at a standstill. When the operating arm has passed the trigger sufficiently to allow that to be re-set the lid may be opened. This, of course, disconnects the gearing.

The general appearance of the machine is shown in Fig. 3. It will be noted that whereas in Fig. 2 the trigger is set and the revolving arm is just making contact with it, in Fig. 3 the trigger has been released and the switch turned off.

A modification of this mechanism allows the machine to be run for any period up to a certain maximum. In this case the trigger is operated by a clip carrying a nut divided lengthwise travelling along a rod which has a screw thread throughout its length. Reference to Fig. 4 will make the mechanism clear. A is a worm wheel attached to the centrifuge spindle which projects through the hole in the centre of the lid. B is a gear wheel operated by it. C, D, E and F are bevelled gears giving rotation to the threaded rod G. The reduction in speed of this rod depends upon the gearing between A and B, and E and F. E and F may, if still slower revolution of G is required, be replaced by worm and gear wheels as A and B.

H represents the clip which operates the trigger M and it is shown in profile at H.1. K represents a rod against which the clip (H) rests and it is thus prevented from revolving with G and forced to travel along the rod and operate the trigger M. The trigger mechanism is similar to that already described.

It may be pointed out that all the parts used in making these mechanisms are standard " Meccano " units.

ANNOTATION

The Treatment of War Wounds

The subject of the treatment of war wounds is again receiving prominence in the editorial columns of the medical Press. (*Brit. Med. J.*, July 6th, 1940; *Lancet*, July 13th, 1940). This has been stimulated by the recent appointment by the Medical Research Council of a committee " to advise on the application of the results of research to practice in the treatment of war wounds and on the need for new investigations."

During the past 25 years great advance has been made in the technique of wound treatment—the practices of excision and *débridement* of damaged tissues, and the enforcement of rest for the injured part by the application of a plaster of paris cast, are examples. Methods of active immunisation against tetanus have been developed and more potent antitoxic sera to combat anaerobic infections have been produced.

Of recent years chemotherapy in the treatment of wound infection has received a great fillip through the introduction of the sulphonamide preparations. Amongst the subjects which will, no doubt, receive special study are: the merits of the respective compounds in combating the various types of wound infection; their activity as a prophylactic as well as a curative measure and the best routes of administration and methods of local application. To obtain satisfactory evidence it is necessary that this work shall be carried out *in the field*. War wounds are vastly different from the wounds encountered in civil practice and also from the conditions pertaining in animal experiment. Those caused by high explosive bomb and shell fragments are characterised by gross tissue injury and disruption, factors which greatly reduce or completely overcome the natural anti-bacterial powers of the tissue cells, and thus allowing bacterial destruction to occur at a rate and to an extent seldom observed under civil conditions. The surgical treatment of war wounds is based on the principles of excision of damaged tissue, effective drainage and immobilisation. But discretion is urged by the *Lancet* in the application of excision: " the blessed word *débridement* which should mean removal of the damage, the whole damage and nothing but the damage, can cover anything from a very sketchy surgical toilet to a destruction of normal tissues out of all proportion to the needs of the case." Those of us who deal with the treatment of wounds in animals would echo this need for the exercise of discretion, particularly as regards excision of the skin. We are accustomed to dealing with grossly infected wounds and realise that if healing is to occur in a reasonable time, and, often, without deformity, as much skin as possible must be conserved. The *Lancet* states, " well established though these surgical principles may be, it is clear that they need to be repeated to the point of boredom. Men have lately been received in the French hospitals and our own whose wounds have been sutured two, three and even four days after infliction. The severe local infection, spreading cellulitis, gas gangrene and not uncommon death that may follow this neglect of simple pathological teaching must be known if a repetition of this tragedy is to be avoided." It can be

said with truth that the veterinary surgeon has long been aware of the basic surgical principle that all accidental wounds are infected ones and that their complete closure by suture is bad practice. Even when disastrous results such as those outlined above do not occur it is often realised that the operation was a waste of time for in a matter of 48 hours or so it is imperative to remove all or the greater part of those carefully inserted sutures to provide drainage for pus. It is recognised that a few sutures may with advantage be introduced to prevent sagging and to bring wounded tissues into closer adaptation, provided always that there is free drainage from the depth and dependent parts. Not infrequently the introduction of sutures into bruised skin is the factor which is responsible for the subsequent necrosis of that skin. Yet the public is "suture minded." It tells proudly of the number of sutures, generally high, to which its wounds have been subjected, and one can only conclude that this is the outcome of its medical experience. The veterinarian is frequently chided for his apparent neglect of duty in failing beautifully to sew up an infected wound. Surgical teaching would do well to postulate that the proper treatment of an accidental wound is to leave it open, suturing being an exceptional procedure only adopted after the most careful consideration, and not the converse.

J. G. W.

ABSTRACTS

[**ANASTOMOSIS OF THE INTESTINE.** Stewart, G. M. (1939.) *Can. J. comp. Med.* **3**. 258-259. (7 figs.).]

An end-to-end method of bowel anastomosis is described, the technique of which is as follows :—

The intestine is exposed and protected with sterile swabs. Contents of the bowel are forced back with the fingers and thumb for about two inches above and below the disclosed portion and clamped with forceps. The mesentery is incised and any vessels ligated. All diseased bowel is excised and the lumen of the bowel as far as the clamp on either side is swabbed out with some mild antiseptic (Fig. 1).

A round probe is inserted into section A and an incision half an inch long is made about one inch from the severed end (Fig. 2).

The probe is removed and forceps are passed through the incision to grip the cut edge of the intestine and gentle traction inverts it (Figs. 3 and 4).

Forceps are now introduced through the lumen of the inverted piece of bowel and after passing through the incision grasps the divided end B and pulls it through into the inverted part of section A so that the ends of each coincide (Figs. 5 and 6).

The ends of sections A and B are sutured together with a continuous Lembert suture, using fine silk.

Section B is now gently pulled back through the incision which is closed with Czerny-Lembert sutures (Fig. 7).

The clamps are removed, the bowel replaced in the peritoneal cavity and the abdominal wounds closed.

The advantages of this method over all others claimed by the author are that the technique is simple, the anastomosis is more secure and there is less chance of leakage.

The abstractor has not had the opportunity of putting the method into practice on the living subject but performed on the cadaver the technique is ingenious and seems full of promise.

C. F.

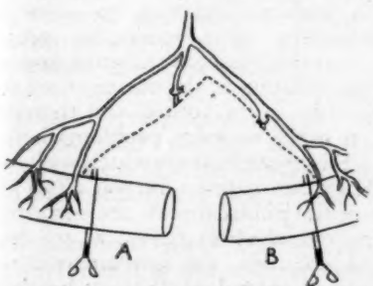
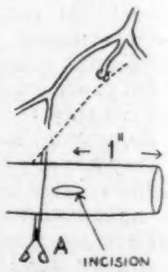


FIG. 1



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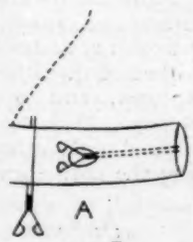


FIG. 3

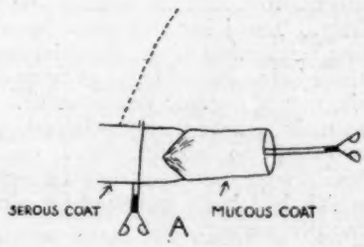
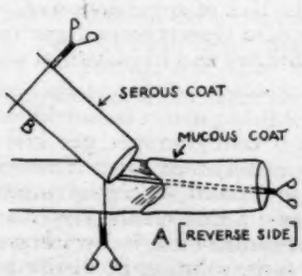


FIG. 4



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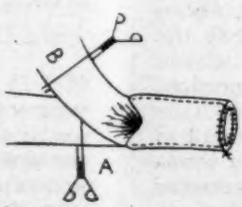

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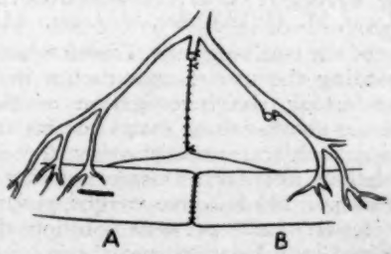

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PERSONAL

Marriage.—CARNEGIE—BOON.—On September 19th, at the Parish Church, Kendal, William Kemp Carnegie, B.Sc., M.R.C.V.S., second son of Mr. and Mrs. W. Carnegie, Airth, Falkirk, to Marjorie Webster Boon, only daughter of Mr. and Mrs. F. W. Boon, Eller Lea, Kendal.

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WAR DAMAGE TO PROPERTY

In his recent statement to the House of Commons, the Prime Minister said that the damage to property by enemy action to date was very different from the estimate given to the War Committee which considered and decided against the possibility of an insurance scheme against such damage. It would, in his judgment, be worth while to make a further examination of such a scheme, particularly as it would affect the small man. He had therefore asked the Chancellor of the Exchequer o consider the best way of making such a review in the light of the facts as they were then.

In the meantime, continued Mr. Churchill, the Chancellor of the Exchequer had agreed to the following arrangements, in addition to the satisfactory provisions which had already been made in respect of the personal injuries and immediate needs of those smitten. At present in cases where the income of the claimant's household did not exceed £400 a year and his resources were limited, payments were made to cover damage to essential household furniture up to a maximum of £50, and similar payments were made in respect of personal clothing up to £30, subject to income limits of £400 where there were dependants and £250 where there were no dependants. It was now proposed to abolish these upper limits of £50 and £30 respectively, so that payments for damage to the furniture or clothing of persons of limited means would now be made up to 100 per cent. of the damage, whatever that amount might be.

Similar payments would be made to professional people within the same limits of income.

* * * * *

CASUALTY LIVESTOCK

The Ministry of Food has issued an instruction to slaughterhouse managers that in future the owners of any casualty animal must, if he so desires, be allowed to see the carcase of his animal graded and weighed. Unless he has exercised his option of being present, the owner must be notified at once by the slaughterhouse manager whenever the carcase or part of the carcase of a casualty animal is condemned. Such carcase or part of a carcase will be held available at the slaughterhouse for inspection by the owner for 24 hours after condemnation.

* * * * *

FOOT-AND-MOUTH DISEASE IN YORKSHIRE

The existence of foot-and-mouth disease was confirmed on Friday of last week among cattle and pigs at Wibsey, Bradford. The area subject to restrictions in the movement of livestock lies within the counties of Lancashire and the West Riding of Yorkshire.

* * * * *

COSTLY DRUGS AND THE PURCHASE TAX

The Treasury gives notice that under the provisions of the Finance (No. 2) Act, 1940, it is proposed to lay before the House of Commons the following draft list more particularly defining "essential drugs of an exceptionally costly character" which shall be exempted from Purchase Tax:—

Arsphenamine and its derivatives, bromethol, chloroform (anaesthetic), cocaine and salts of cocaine, desoxycorticosterone acetate, ether (anaesthetic), ethyl chloride, hexabarbitone, insulin, liver extract and active principles of liver, hormones, natural or synthetic and preparations thereof, including liver extracts and active principles of liver, and insulin and its compounds with protamin and zinc; oxygen, pituitary extract, procaine and salts of procaine, sulphanilamide and its derivatives, suprarenal cortex extract, sera, vaccines.

Any representations in regard to this list should be addressed in writing direct to the Commissioners of Customs and Excise, Custom House, Lower Thames Street, E.C.3, not later than October 1st.

* * * * *

The National Farmers' Union has moved its headquarters to 478, West Wycombe Road, High Wycombe, Bucks.

* * * * *

A RATIONAL APPROACH TO RESEARCH IN CHEMOTHERAPY

To the issue of the *Lancet* of May 25th, Paul Fildes, Director of the Department of Bacterial Chemistry, Medical Research Council, contributes under the above heading a valuable review, having special reference to the factors requisite for both growth and inhibition of bacteria.

Introducing his observations with a review of the findings of authors who conclude that the action of sulphanilamide *in vivo* depends on an interference with some bacterial enzyme reaction which is necessary for the growth of the organism, Fildes makes reference to the obtaining by Woods (1940) of an extract from yeast which counteracted the action of sulphanilamide and to his identification of the active material as *p*-aminobenzoic acid. "This substance is looked upon as an 'essential metabolite' with which sulphanilamide interferes as will be discussed later. These authors are thus in agreement in ascribing the action to interference with bacterial enzymes associated in some way with nutritive substances."*

Dr. Fildes continues:

"The wider aspects of inhibition of bacterial growth have been the subject of study in this department for a number of years, following as a natural sequence on our studies of bacterial 'growth factors,' and evidence has been accumulating that inhibition of bacterial growth in general may be exerted through interference with one of these 'factors.' Since the definition of a growth factor has tended to become modified it will be necessary for a proper understanding of the theory of interference to outline our present views on this subject.

Bacterial Growth Factors

"The growth and multiplication of bacteria (and no doubt of all cells) involves the synthesis of new bacterial substance from simpler compounds. In the case of protein the simplest compound is ammonia and from this and a suitable source of carbon some bacteria can synthesise protein, the energy required in the process being liberated from a combustible material such as glucose. Although little or nothing is known about the mechanism of this synthesis, it is inferred that it is achieved in steps by a series of enzymes operating on each stage as it is completed. . . . Each stage in any synthesis necessary for growth is referred to as an 'essential metabolite,' without which, either synthesised or supplied from outside, growth cannot occur. A growth factor is thus an essential metabolite which cannot be synthesised. The requirement by a particular species for an essential metabolite to be given as a growth factor is not characteristic for the species. Some strains can synthesise it more or less or can be induced to do so. At the moment the view is gaining ground that the essential metabolites at present known in bacteriology, or at any rate many of them, are common to all or most cells whether bacterial or not.

"Although the process of growth has been represented as a building up of bacterial protoplasm, there are other processes to be carried out subsidiary to this main object.

*Fleming (1940) showed that suspensions of a number of bacteria in a solution of sulphanilamide, even when dead, counteracted the action of the drug. The effect was due to substances given off by the bacteria under these conditions.

For instance, the process by which energy is set free must be catered for. This involves the synthesis of various co-factors which take part in the process. Thus cozymase, a substance of known composition containing nicotinic acid, is a constituent probably of all cells and is associated with the process by which glucose, for instance, is combusted. . . . Cozymase is an essential metabolite for *Bact. coli, Proteus vulgaris, Bact. typhosum* and *Haemophilus influenzae*, but it is a growth factor only for *H. influenza*, while nicotinic acid, a stage in the synthesis of cozymase, is a growth factor for proteus and staphylococcus. These relationships are indicated in the figure:—

Synthesis of Cozymase

NH_3 —> Amino-N (Stage 1) —> Nicotinic acid (Stage 2) —> Cozymase (Stage 3)

Essential metabolite—
Stage 1 = growth factor for *Bact. typhosum*.
„ 2 = „ „ staphylococcus and proteus.
„ 3 = „ „ *H. influenza*.

"It will, of course, be understood that the direct discovery of an essential metabolite can only be made in the case of a bacterium for which it is a growth factor—*i.e.*, which cannot synthesise it. In addition to the many growth factors which have been described already, it is presumed that there are others which cannot be detected until a bacterium is found which cannot synthesise them.

"Bacteria (and other cells) are thus looked upon as organisms which are potentially able to synthesise their structure and accessory reagents from simple compounds, though many as a result of altered environment have more or less lost the power to carry out one or more stages of essential syntheses (cf., Lwoff 1932, Knight 1936). In such cases the particular stage of synthesis which fails becomes a growth factor which must be added to an artificial nutrient mixture or borrowed from a host which has either synthesised it itself or ingested it in its nutrients. Whether, however, an organism can synthesise the stage or not it remains an essential metabolite, interference with which will inhibit growth.

Mechanism of Bacterial Inhibition

"The evidence on which we have been led to associate bacterial inhibition with interference with essential metabolites is broadly as follows.

"Fildes (1929) and Knight and Fildes (1930) showed that anaerobes would not grow if the oxidation-reduction potential of the medium was maintained positive to a certain level. In less technical language this implies that anaerobes will only grow when a certain degree of reducing intensity is present in the medium. If the degree of reducing intensity is diminished by oxygen or by an oxidising agent such as ferricyanide, thionine or methylene-blue no growth takes place until these have been reduced. The metabolism of an anaerobe is 'anaerobic'—*i.e.*, it involves the presence of reduced substances in the nutrients or at least an absence of oxidising substances. A similar inhibiting effect of thionine or methylene-blue is also observed with aerobes, for the reason that conditions *within* an aerobic cell are anaerobic and a sufficient concentration of these diffusible dyes imposes aerobic conditions within the cell.

"This subject was discussed by Fildes (1937), who pointed out that the inhibition was only observed with diffusible dyes and not with non-diffusible dyes of a similar oxidising capacity. It was concluded that the inhibition was due to oxidation of some substance within the cell, which in metabolism required to be reduced. In more recent phraseology it was due to an interference by oxidation with an essential metabolite which required to be reduced in order that the enzyme associated with it might function normally as in growth.

"At this stage a number of essential metabolites had been isolated, and in particular the group -SH was known to be necessary for staphylococci (Fildes and Richardson 1937) and moulds (Volkonsky 1932). In view of the fact that H-SH combines with mercury, and H-SH reverses the antibacterial action of mercury was it possible that the antibacterial action of mercury was due to combination with essential -SH groups within the cell? This subject has been dealt with recently by Fildes (1940). He showed that mercury combined in the test-tube with -SH in certain molecular proportions to form a soluble compound devoid of -SH. This compound was not in itself inhibitory. Inhibition was only observed when mercury in a mixture of mercury and -SH was in excess of the molecular proportions noted. It was concluded that the antibacterial action of mercury was due to the diffusible salt entering the cell, thus depriving it of an essential metabolite. And in the concentrations used this was the only action of mercury, since addition of excess of -SH allowed the inhibited organisms to grow normally—*i.e.*, excess of the essential metabolite did away with the inhibition.

Mode of Action of Sulphanilamide

"With the introduction of the sulphanilamide drugs, it became a question whether the same argument could not be applied and in this matter we were able to profit by the experience of McIntosh and Whitby. Prolonged experiments were carried out to test whether any known essential metabolite could be associated with the action of sulphanilamide, in the sense that a quantitative relationship could be shown to exist between the inhibitory concentration of sulphanilamide and the counter-action of the essential metabolite. These experiments were unsuccessful. At this stage Stamp (1939) showed that he was able to reverse the action of sulphanilamide by an extract obtained from streptococci. We assumed at once (as also did Stamp) that this extract contained an essential metabolite which was not a growth factor for streptococci, since at this time all these were known (McIlwain 1940). As has been explained we do not look upon essential metabolites as specific to one species of bacterium or even to bacteria at all, and thus for convenience made extracts from yeast following more or less the procedure of Stamp. The result of this work has been published by Woods (1940). By fractionation of yeast extracts he obtained a material with well-defined chemical characters which reversed the action of sulphanilamide. This material could not for quantitative reasons combine with sulphanilamide on a molecular basis as does -SH with mercury, but it might act by competing with sulphanilamide for an enzyme of which it was the natural substrate. In this case it should follow that the substance in the extract had some structural similarity with sulphanilamide, and it became a question as to what substance similar to sulphanilamide had the chemical characters of the extract substance; *p*-aminobenzoic acid was tried and was found to antagonise sulphanilamide quantitatively and in very high dilutions. At the moment, therefore, it is supposed that *p*-aminobenzoic acid is an essential metabolite for bacteria, although for technical reasons it has not yet been isolated from them nor has it yet been proved to be a growth factor in the absence of a bacterium which cannot synthesise it. This essential metabolite is normally associated with an enzyme, and sulphanilamide, being structurally similar to it, is capable if in sufficient concentration of displacing *p*-aminobenzoic acid from its enzyme and stopping this essential line of metabolism.

"On this view the 'sensitivity' of a microbe to sulphanilamide would depend at least in part upon whether it could synthesise *p*-aminobenzoic acid readily or not. An organism whose synthetic powers were poor would be more sensitive than one with greater power. Similarly a large number of bacteria would be less affected by a certain concentration of sulphanilamide than a small number. Inhibition or not would become a question of the proportion of sulphanilamide to *p*-aminobenzoic acid affecting the enzymes of each cell.

"It is of interest that *p*-aminobenzoic acid also antagonises sulphanilamide *in vivo* (Selbie 1940), so that animals die from a streptococcal infection in the presence

of a curative dose of sulphanilamide when *p*-aminobenzoic acid also is added.

"Since, in our view, *p*-aminobenzoic acid is an essential metabolite not only for bacterial cells but also for animal, curative interference with it *in vivo* will affect both bacterium and host. Success in treatment must then depend upon the relative "sensitivity" of the two types of cell or upon whether the host as a whole can survive the inhibition of this enzyme reaction for sufficiently long. It is possible that this inhibition may account for some of the later toxic effects of sulphanilamide. These effects might then be looked upon as due to a deficiency in *p*-aminobenzoic acid and might be treated by administration of this deficient substance.

Line for Future Research

"If further work confirms the conclusion drawn from experiments with the three different antibacterial agents, dyes, mercury and sulphanilamide—that the antibacterial actions are due to specific interferences with essential metabolites—it should follow that an antibacterial substance should be capable either of combining with an essential metabolite to form a product devoid of the essential metabolic function, or of blocking an enzyme specifically associated with the metabolite. In this latter case it is to be expected that the antibacterial substance should have a chemical similarity to the essential metabolite. Chemotherapeutic research might then reasonably be directed to modification of the structure of known essential metabolites to form products which can block the enzyme without exhibiting the specific action of the metabolite. It is curious to note that chemists are already to some extent proceeding along these lines. In sulphapyridine, for instance, the base attached to the sulphanilamide is related to nicotinic acid, and in sulphathiazole to vitamin B_1."

CORRESPONDENCE

Letters to the Editor should reach the Office not later than by the first post on Tuesday morning for insertion in following Saturday's issue.

The views expressed in letters addressed to the Editor represent the personal view of the writer only and must not be taken as expressing the opinion or having received the approval of the N.V.M.A.

* * * * *

ETIOLOGY OF EQUINE SPECIFIC OPHTHALMIA

To the Editor of the Veterinary Record

Sir,—One of the penalties of exile is that one can only join in a discussion in the *Record* long after most people have forgotten what it was about. This letter refers to a paper on equine specific ophthalmia read by Mr. T. L. Wright at the Conway Hall on April 4th, and discussed by Lt.-Col. Greenfield, Mr. W. S. King and others—*vide Veterinary Record* of May 25th, 1940.

Your editorial article stressed the main conclusion to be drawn from a good paper and a full discussion: no one seemed to know anything about the cause of the disease. This surprised me, because it is stated in the chapter on "Diseases" in the "Official Veterinary History of the Great War" that Dalling claimed to have isolated a causal organism. Moreover, it was generally agreed at the time from a great deal of evidence that the essential cause of the disease—bacterium or virus—was contained in the soil: it was obviously a soil-borne disease. That is not to say that there was any particular area which, without the circumstances of war, contained in infective quantity and quality the first cause of specific ophthalmia. Time and animal-congregation, with long and intense fouling of the soil in certain areas, were needed for infection.

On such grounds it is easy to understand why about two years had to pass before the disease appeared, both in the Boer War and the Great War. In South Africa by December, 1901, the earth of the horse-lines in veterinary hospitals, remount depots and standing camps had been so thoroughly saturated with equine urine and made so completely foul that the normal bacterial flora, and the chemical reaction of the soil, must have been changed. Exactly the same changes had occurred in those parts of France occupied by our horses and mules in 1916. With the end of the war and the consequent disappearance of these intensive conditions specific ophthalmia also more or less entirely disappeared. Those of us who served for a year or more in South Africa as veterinary officers to a flying column in charge of about a thousand horses and mules never saw a case of specific ophthalmia, but when the war ended and we were posted to duty in the befouled standing camps we saw all sorts of gross infection including much ophthalmia. But not everywhere: some areas in Natal seemed free from the disease. My old South African War comrade, Lt.-Col. Knott, had this difference in mind when he advised the Central Meeting to "keep your horses out in the open and you will not get specific ophthalmia."

Mr. Wright said that specific ophthalmia had disappeared from South Africa, but last year, in consultation with Dr. Noel Viljoen, M.R.C.V.S., of Bloemfontein, I observed an acute case of this disease in a thoroughbred mare. She had come up after six months at grass in perfect health and was placed in a badly befouled earthen-floored loose-box on the local racecourse. After a few days she went off her food and showed a slight pyrexia. In another day or two she developed a typical and very acute iridocyclitis of one eye and in a few more days the other eye was affected. The first eye became disorganised and she lost the sight of it: the other eye cleared up completely.

I am satisfied that this disaster would not have occurred if either the mare had been kept at grass or had been placed in a surgically clean loose-box. The combination of metabolic change and an earth standing befouled by decades of urine favoured an adverse bacterial or virus action that would not have occurred in the absence of these conditions.

Like equine shipping fever, which cannot be experimentally reproduced in single instances, specific ophthalmia is a disease requiring for its creation equine crowding, metabolic disturbance, with—in addition—a certain sort of soil long befouled with equine excreta.

As regards treatment, I was interested to read Mr. Wright's reliance on potassium iodide in the drinking water. Major Frank Chambers, in France in the Great War, claimed good results from injecting tincture of iodine above the eyeball: if iodine has a specific curative effect it might act as well when administered in that way as when given by the mouth.

Yours faithfully,
Wakefield Rainey.

c/o Union Bank of Australia,
Hobart,
Tasmania.

July 15th, 1940.

* * * * *

INJURED TEATS IN COWS

To the Editor of the Veterinary Record

Sir,—Cases of teat injury involving the teat canal are commonly experienced as a result of tears from barbed wire, etc., and subsequent treatment usually results in stricture of the canal and mammary trouble.

I believe on the continent amputation of the teat at the base is practised; possibly some of your readers can detail experience of this treatment, together with the technique employed.

I may say that in South Africa it is usually impossible, on the grounds of expense, to follow up cases with subsequent visits, and the ordinary treatment of suturing the wounds is most unsatisfactory.

Yours faithfully,
F.R.C.V.S.

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